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ARTICLE

Resonance assignments for the substrate binding domain of Hsp70 chaperone Ssa1 from *Saccharomyces cerevisiae*

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Abstract Hsp70 chaperone proteins play crucial roles in the cell. Extensive structural and functional studies have been performed for bacterial and mammalian Hsp70s. Ssa1 from *Saccharomyces cerevisiae* is a member of the Hsp70 family. In vivo and biochemical studies on Ssa1 have revealed that it regulates prion propagation and the cell cycle. However, no structural data has been obtained for Ssa1 up to now. Here we report the almost complete (96 %) ^{1}H, ^{13}C, ^{15}N backbone and side chain NMR assignment of the 18.8 kDa Ssa1 substrate binding domain. The construct includes residues 382–554, which corresponds to the entire substrate binding domain and two following α-helices in homologous structures. The secondary structure predicted from the assigned chemical shifts is consistent with that of homologous Hsp70 substrate binding domains.

Keywords Ssa1 · *Saccharomyces* · Substrate binding domain · NMR assignments · Secondary structure

Biological context

Hsp70 constitutes a highly conserved family of molecular chaperones, existing ubiquitously in all organisms and cellular compartments (Zuiderweg et al. 2013). Hsp70 is considered a core mediator of protein homeostasis, because of its various roles in processes such as protein folding, protein degradation, translation and translocation (Zuiderweg et al. 2013).

The Hsp70 family shares a highly conserved architecture, consisting of an N-terminal nucleotide binding domain (NBD, also termed the ATPase domain), a substrate binding domain (SBD) containing a hydrophobic substrate-binding pocket, and a C-terminal lid domain (CTD), which caps the hydrophobic pocket and regulates substrate binding (Zuiderweg et al. 2013). Allosteric communication between the NBD and the SBD/CTD has been intensively studied, as recently reviewed: N-terminal ATP hydrolysis induces a conformational change in the NBD, which then induces a conformational change in the SBD, enhancing its substrate binding ability, while binding of substrate to the SBD also stimulates the ATPase activity of the NBD (Zuiderweg et al. 2013).

The Ssa subfamily of Hsp70 chaperones comprises four members (Ssa1–4) and is essential for cell viability (Wernerwashburne et al. 1987). Ssa1 is involved in yeast prion propagation, and its overexpression, deletion and mutation often affect stability of yeast prions (Liebman and Chernoff 2012). The mutation L483W in the SBD of Ssa1 was found to impair [*PSI*$^{+}$] propagation and weaken the phenotype caused by [*PSI*$^{+}$] (Jung et al. 2000). Due to lack of an available structure for Ssa1, the DnaK SBD structure was used to perform steered molecular dynamics on mutants of Ssa1 that affect prion propagation in vivo (Xu et al. 2013a); and DnaK and bovine Hsc70 structures were used to obtain a model structure of full-length Ssa1 in order to carry out a mutagenesis study of the structural basis for inhibition of amyloid formation of a yeast prion protein in vitro (Xu et al. 2013b). Ssa1 can interact with yeast prion

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proteins directly (Shorter and Lindquist 2008; Xu et al. 2013b). It was observed that the SBD of Ssa1 alone is sufficient to inhibit Ure2 fibril formation, but the presence of the CTD enhances this inhibition (Xu et al. 2013b). Ssa1 also cooperates with other chaperones including Hsp104, Ydj1, Sis1, Fes1, and Sti1 to modulate prion propagation (Shorter and Lindquist 2008). Other studies show that post-translational modification (PTM) of Ssa1, namely phosphorylation at T36 in the NBD, controls G1 cyclin abundance and cell-cycle progression (Truman et al. 2012). A

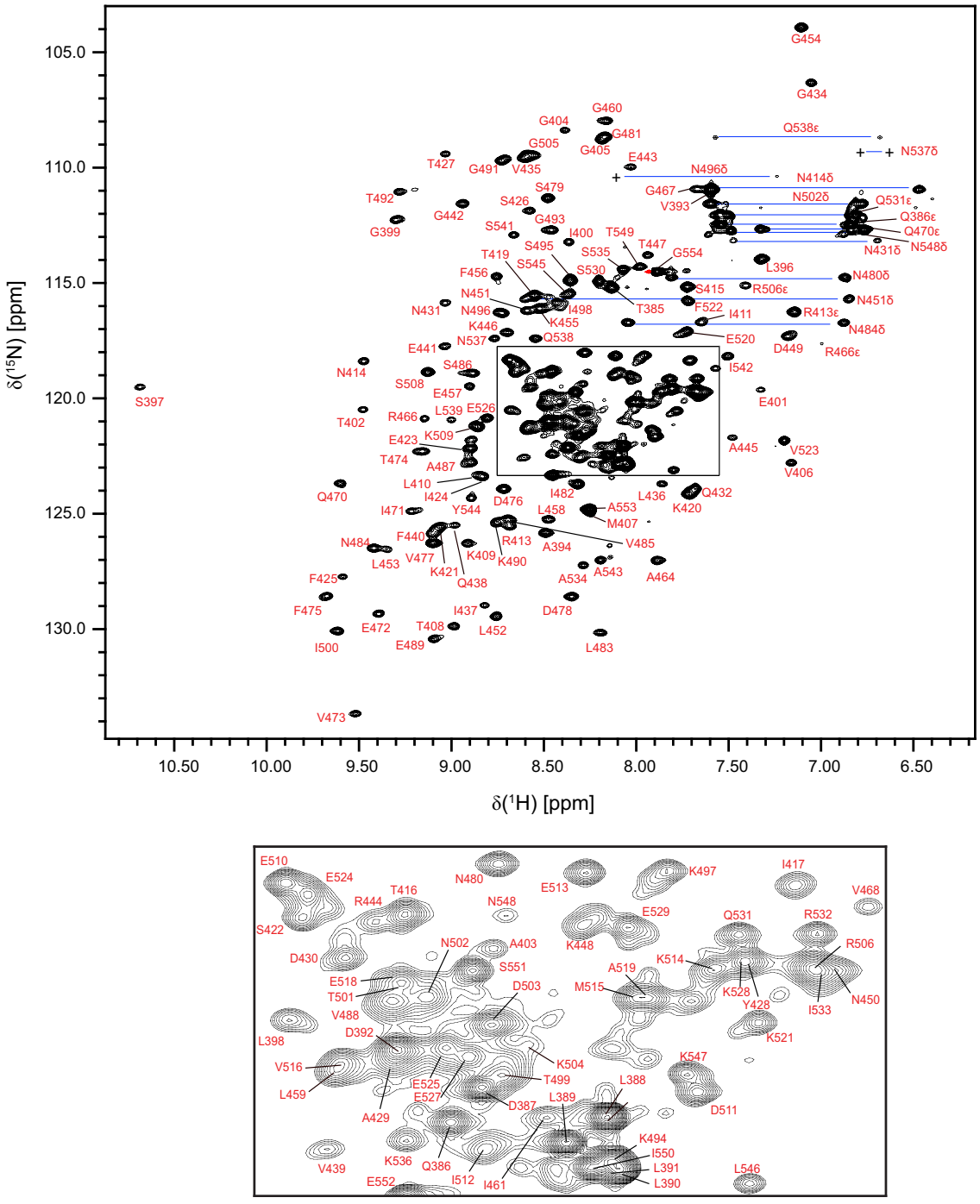

Fig. 1 2D ^{1}H–^{15}N HSQC spectrum of Ssa1 SBD at 298 K. Assignments are indicated by the *one-letter* amino acid code and the *sequence number*. Side chain NH signals from N496 and N537 are under the plot contour level and their positions are indicated by *crosses*. There are some unassigned weak peaks in the HSQC spectrum of the Ssa1 SBD, which are possibly due to slight local conformational heterogeneity

number of other PTM sites have been identified in the NBD, SBD and CTD of Ssa1 (Beltrao et al. 2012; Henriksen et al. 2012). Structural studies are necessary in order to understand the possible regulation mechanisms of different types of PTM.

Although many functional studies have been performed on yeast Ssa1, there is only limited structural data available for the yeast Ssa family, as most structural studies have focused on bacterial Hsp70 (DnaK) and mammalian Hsp70. Thus, structural investigation of Ssa1 will provide information that is important to understand related functional and mechanistic studies. Here, we report the complete ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the truncated SBD domain of Ssa1 from *Saccharomyces cerevisiae*, which includes residue 382–554 of the full length protein. This serves as a starting point for structure determination and continued functional studies of the Ssa1 SBD domain.

Methods and experiments

Protein expression and purification

The truncation mutant of Ssa1 SBD (corresponding to amino acid residues 382–554 of the full length protein) was cloned into a modified pET28a plasmid containing an N-terminal 6×His tag and a SUMO tag (Zheng et al. 2012). The plasmid was used to transform *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells. After induction by IPTG, ^{15}N and $^{15}N/^{13}C$ labelled recombinant proteins were expressed in the transformed cells grown in ^{15}N–NH_4Cl and ^{13}C-glucose enriched M9 minimal medium.

The expressed proteins were first purified by Ni affinity column (Chelating Sepharose Fast Flow; GE Healthcare), followed by SUMO protease Ulp1 treatment (2 h at 4 °C) to cleave the N-terminal 6×His tag and SUMO tag. The protease cleavage leaves an extra serine as the N-terminal residue of the protein of interest. An additional Ni affinity chromatography step was applied to remove uncleaved protein, the digested N-terminal SUMO tag and Ulp1 protease. The proteins were further purified by gel filtration chromatography using a Superdex^{TM} 75 column (GE Healthcare).

NMR spectroscopy

NMR samples consisted of 1 mM protein in 50 mM NaH_2PO_4–Na_2HPO_4, 50 mM NaCl, 1 mM DTT, 1 mM EDTA and 10 % (v/v) D_2O at pH 7.0. All NMR experiments were performed at 298 K on a Varian INOVA 600 MHz spectrometer equipped with a triple resonance cryo probe. ^{1}H–^{15}N and ^{1}H–^{13}C HSQC, HNCO, HN(CA)CO, HNCACB and CBCA(CO)NH spectra were recorded for backbone assignment. The side chain assignments were based on C(CCO)NH, HBHA(CO)NH, HCCH-TOCSY, CCH-TOCSY, ^{1}H–^{15}N and ^{1}H–^{13}C NOESY-HSQC experiments. All spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed with NMRViewJ (Johnson and Blevins 1994).

Assignment and data deposition

The Ssa1 SBD construct (residues 382–554) contains 174 amino acids, including the N-terminal serine left behind by protease cleavage. The ^{1}H–^{15}N HSQC spectrum of the Ssa1 SBD and the assignment of the amide proton signals are shown in Fig. 1. All non-proline backbone amide protons and nitrogen signals of the Ssa1 SBD could be assigned, except for the signals of the N-terminal serine and Ser582–Lys584, Leu507 and Leu540. Assignment is missing for all atoms of Ser582–Ser583, while assignments were obtained for Hα, Cα and the side chains of Lys584, Leu507 and Leu540. The assignment of the backbone resonances of H^N, N, C^{α} and C^{β} was completed to 98 %. Assignment of aliphatic and aromatic side chains was achieved to 95.7 % (side chain atoms of lysine residues, OH, SH, side chain $^{13}C'$, $^{13}C^{\xi}$, and quaternary ^{13}C were excluded). There are some unassigned weak peaks in the ^{1}H–^{15}N HSQC spectrum of the Ssa1 SBD, which are possibly due to slight local conformational heterogeneity.

The secondary structure of the Ssa1 SBD was predicted from the assigned chemical shifts using the software TALOS+ (Shen et al. 2009). The results indicate that the

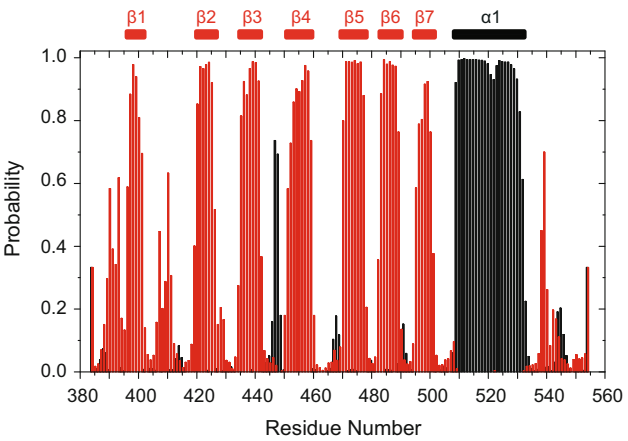

Fig. 2 The secondary structure of the Ssa1 SBD predicted by the software TALOS+ (Shen et al. 2009). The predicted α-helix and β-sheet probabilities of each residue are plotted in *black* and *red*, respectively. The secondary structure elements, indicated on the *top* of the figure, were obtained with the criterion that the probability >0.5 for three or more continuous residues

Ssa1 SBD contains one α-helix and seven β-strands in the order β1–β2–β3–β4–β5–β6–β7–β8–α1 (Fig. 2). The predicted secondary structure is consistent with the human homologous structure (PDB ID: 1CKR) (Morshauser et al. 1999). The structure determination of the Ssa1 SBD based on the reported chemical shift assignments is underway. All assigned chemical shifts were deposited in the BioMagResBank of the University of Wisconsin-Madison under the accession number 25385.

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